Current Biology, Vol. 13, R976–R978, December 16, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/j.cub.2003.11.048

Calcium Influx: Is Homer the Missing Link?

Dispatch

H. Llewelyn Roderick and Martin D. Bootman

Calcium signals in cells can arise via release from intracellular stores or influx across the plasma membrane. Recent studies have shed new light on the multi-protein signalling complexes that mediate communication between calcium stores and plasma membrane calcium channels.

Cytosolic calcium signals occur in response to diverse stimuli and regulate a range of cellular processes [1]. In most non-excitable cells, increases in cytosolic calcium are brought about by its release from the endoplasmic reticulum (ER). The principal release channels are inositol 1,4,5-trisphosphate (IP_3) receptors and ryanodine receptors, although other calcium channels may participate [1]. The cytosolic calcium concentration may also be increased by influx across the plasma membrane, although the mechanism by which this occurs in non-excitable cells is only just becoming clear. Calcium entry into a cell serves two purposes: it can generate specific calcium signals for certain cellular processes, and it can replenish depleted intracellular stores [2].

Emptying the ER calcium stores activates calcium entry, a process generally known as store-operated calcium entry [2]. The mechanism by which depletion of ER calcium activates channels in the plasma membrane, however, is unclear. The most prominent model proposes a conformational coupling between IP_3 receptors on the ER and influx channels on the plasma membrane [3]. In this scheme, reduction of the ER luminal calcium concentration provokes a conformational change in IP_3 receptor, which is physically linked to the activation of store-operated calcium entry channels.

It has been suggested that mammalian orthologues of the *Drosophila* transient receptor potential (TRP) proteins form the calcium influx channels [2]. In *Drosophila*, TRPs are responsible for the light-induced current that underlies phototransduction. The overproduction or ablation of mammalian canonical TRP isoforms (TRPCs) has been reported to modulate store-operated calcium entry. However, not all TRPC isoforms — of which seven are presently known — are activated solely by depletion of ER calcium stores. The role of TRPCs is thus still contentious [4,5], but they remain the most promising candidate mediators of store-operated calcium entry.

All TRPC and IP_3 receptor isoforms can physically interact [6,7], which strengthens the case that conformational coupling underlies the activation of store-operated calcium entry. The site of interaction has been mapped to the carboxyl terminus of TRPC3 and the amino terminus of the IP_3 receptor, both regions that are conserved among all isoforms of the respective proteins [7]. Disrupting this interaction can prevent store-operated calcium entry. For example, induction of a cortical actin ring around the periphery of a cell [8], or overproduction of a dominant-negative form of SNAP-25 which blocks secretion [9], inhibits store-operated calcium entry. These findings have been taken to support a 'secretion-coupling' model in which the TRP–IP_3 receptor complex is not preformed, but is produced by reversible translocation of IP_3 receptors following depletion of the ER calcium stores. But while some studies have demonstrated an increase in TRP–IP_3 receptor interaction following calcium store depletion [10,11], others have not. So whether TRP–IP_3 receptor complexes are constitutively present or transiently formed is not known, but they do seem to be required for store-operated calcium entry.

The TRP proteins responsible for phototransduction in *Drosophila* are known to be part of large complexes formed by multiple protein molecules linked together into a functional 'signalplex'. In *Drosophila*, the signalplex includes orthologues of protein kinase C (PKC), phospholipase C (PLC), rhodopsin and TRPs, held together by the PDZ domain-containing 'scaffold' protein InaD [12]. Proteins containing PDZ domains may also play a role in bringing together various components of the calcium signalling toolkit in mammalian cells. For example, the PDZ-domain-containing protein NHERF — Na/H exchange regulatory factor — interacts with the carboxy-terminal amino acids of TRPC4 and TRPC5, with PLCβ and also with the actin cytoskeleton via interactions with members of ezrin/radixin/moesin family [13]. In addition, the carboxyl terminus of TRPC4 can also bind to the scaffold protein EBP50 — ezrin/moesin/radixin-binding phosphoprotein 50 — facilitating its linkage with the cytoskeleton.

Such cytoskeletal interactions may be critical for appropriate targeting of TRPC4 to the plasma membrane [14]. TRPC4 can also impact the cytoskeleton by interacting with protein 4.1N, which links to actin via spectrin. This interaction has recently been shown to be enhanced by store depletion and may connect the ER calcium store to plasma membrane influx channels [15]. The carboxyl terminus of the IP_3 receptor also binds to protein 4.1N, bringing these channels close to the plasma membrane [16]. The cellular location of TRPCs has not yet been definitively established, but it has been shown that they are present in caveolae, where they may associate with many other proteins including SERCA, $G\alpha_{q/11}$, phospholipase Cβ, caveolin-1 and ezrin [17]. The picture that emerges from these studies is that both TRPCs and IP_3 receptors sit at the centre of a web of protein–protein interactions responsible for bringing the channels together at the correct cellular locations.

The interaction of TRPs and IP_3 receptors is regulated by calcium through its binding to calmodulin [18,19].

Laboratory of Molecular Signalling, The Babraham Institute, Babraham, Cambridge CB2 4AT, UK.

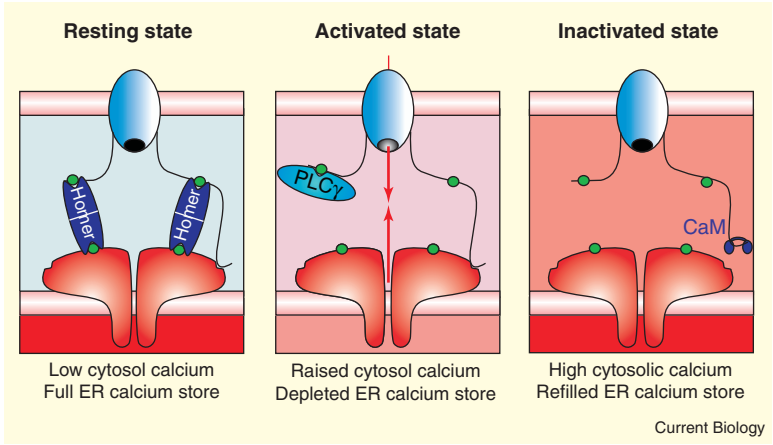

Figure 1. Protein–protein interactions that regulate calcium influx in cells.

Under resting conditions TRPC channels (blue ellipse) are tethered to IP_3 receptors (red channel) directly and via Homer through binding to Homer binding domains (green circle). Store depletion or agonist stimulation results in a disassembly of the Homer–TRPC–IP_3 receptor complex resulting in increased calcium entry (red arrow). During this phase PLCγ may be recruited to the amino terminus of TRPC. Increased cytosolic calcium results in calmodulin (CaM) binding to the carboxyl terminus of TRPC and dissociation of TRPC from the amino termini of IP_3 receptors. During this phase stores refill leading to a return to the resting state.

Two binding sites for calcium–calmodulin have been mapped to the carboxyl terminus of TRPC channels [18], and one overlaps the region where TRPC channels bind IP_3 receptors [19]. Binding of calcium–calmodulin to this site disrupts the TRP–IP_3 receptor interaction thus inhibiting calcium entry. These data support a model where activation of store-operated calcium entry requires TRPC–IP_3 receptor interaction, and competitive disruption of this complex by calcium–calmodulin terminates influx. A similar paradigm may exist for all TRPC isoforms, although the mechanism of calcium–calmodulin inhibition of TRPC activity may differ [18,19].

The interaction of TRPCs and IP_3 receptors is also regulated by Homer proteins [10]. By virtue of their coiled-coil domains, Homer proteins can oligomerise and connect polypeptides bearing proline-rich motifs, as found in IP_3 receptors, ryanodine receptors, metabotropic glutamate receptors and other signalling proteins. A recent study [10] has shown that Homer links TRPC and IP_3 receptors and regulates calcium entry. Homer interacts with both the amino and carboxyl termini of TRPC1 under resting conditions, and in this state it conveys a negative influence on TRPC activation. Depletion of the ER calcium store results in disassembly of the Homer complex and a consequent increase in TRPC channel activity (Figure 1). The physiological relevance of this model was demonstrated in studies on human salivary gland cells, where TRPC1 is reported to be a component of endogenous store-operated calcium entry, and on pancreatic acini from Homer knockout mice.

One member of the Homer family, Homer 1a, does not have a coiled-coil domain, and can disrupt Homer complexes. Homer 1a is the product of an 'immediate early' gene, the expression of which is quickly turned on when cells are appropriately stimulated. Homer 1a disrupts the Homer–TRPC1–IP_3 receptor interaction thus giving an increase in calcium entry, and may therefore function as a dynamic modulator of TRPC–IP_3 receptor complexes.

Apart from Homer, the TRPC interacting proteins discussed thus far interact with the carboxyl terminus of the channel proteins, but their amino termini are also sites of important protein–protein interactions. For example, TRPC channels have four ankyrin repeats, which may provide another linkage of these channels to the cytoskeleton. And TRPC3 and TRPC4 have been shown to require interaction with PLCγ for activity [20]. This interaction was found to be independent of the lipase activity of PLCγ, but to require the enzyme's SH3 domain. Although the region of PLCγ binding has not been precisely mapped, sequence analysis shows that the amino-terminal Homer-binding motif of TRPC1 also conforms to an SH3-binding sequence. As PLCγ interaction and Homer dissociation are required for TRPC activation (Figure 1), reciprocal regulation of TRPC activity by these two proteins is plausible. PLCγ translocation to membranes occurs during calcium mobilization and may thus result in disassociation of Homer from TRPC1 and activation of calcium influx.

The evidence described above suggests that TRPC activity is regulated by a plethora of protein–protein interactions. At present, only the interactions with calmodulin, Homer and PLCγ are understood in any detail (Figure 1). An additional complication arises from the fact that alternative TRPC isoforms appear to have subtly different binding partners and regulation. As these proteins form heteromeric calcium channels — four TRPs give rise to one functional channel — there may be a multitude of signalling inputs to each operational unit, and their regulation *in vivo* is likely very complex.

References

1. Berridge, M.J., Bootman, M.D., and Roderick, H.L. (2003). Calcium signalling: dynamics, homeostasis and remodelling. Nat. Rev. Mol. Cell Biol. *4*, 517-529.
2. Venkatachalam, K., van Rossum, D.B., Patterson, R.L., Ma, H.T., and Gill, D.L. (2002). The cellular and molecular basis of store-operated calcium entry. Nat. Cell Biol. *4*, E263-E272.
3. Irvine, R.F. (1990). 'Quantal' Ca^{2+} release and the control of Ca2+ entry by inositol phosphates — a possible mechanism. FEBS Lett. *263*, 5-9.
4. Ma, H.T., Patterson, R.L., van Rossum, D.B., Birnbaumer, L., Mikoshiba, K., and Gill, D.L. (2000). Requirement of the inositol trisphosphate receptor for activation of store-operated Ca^{2+} channels. Science *287*, 1647-1651.
5. Trebak, M., Vazquez, G., Bird, G.S., and Putney, J.W., Jr. (2003). The TRPC3/6/7 subfamily of cation channels. Cell Calcium *33*, 451-461.
6. Kiselyov, K., Mignery, G.A., Zhu, M.X., and Muallem, S. (1999). The N-terminal domain of the IP3 receptor gates store-operated hTrp3 channels. Mol. Cell *4*, 423-429.
7. Boulay, G., Brown, D.M., Qin, N., Jiang, M., Dietrich, A., Zhu, M.X., Chen, Z., Birnbaumer, M., Mikoshiba, K., and Birnbaumer, L. (1999). Modulation of Ca(2+) entry by polypeptides of the inositol 1,4, 5-trisphosphate receptor (IP3R) that bind transient receptor potential (TRP): evidence for roles of TRP and IP3R in store depletion-activated Ca(2+) entry. Proc. Natl. Acad. Sci. USA *96*, 14955-14960.

8. Patterson, R.L., van Rossum, D.B., and Gill, D.L. (1999). Store-operated Ca^{2+} entry: evidence for a secretion-like coupling model. Cell *98*, 487-499.
9. Yao, Y., Ferrer-Montiel, A.V., Montal, M., and Tsien, R.Y. (1999). Activation of store-operated Ca^{2+} current in *Xenopus* oocytes requires SNAP-25 but not a diffusible messenger. Cell *98*, 475-485.
10. Yuan, J.P., Kiselyov, K., Shin, D.M., Chen, J., Shcheynikov, N., Kang, S.H., Dehoff, M.H., Schwarz, M.K., Seeburg, P.H., Muallem, S., and Worley, P.F. (2003). Homer binds TRPC family channels and is required for gating of TRPC1 by IP(3) receptors. Cell *114*, 777-789.
11. Rosado, J.A., Brownlow, S.L., and Sage, S.O. (2002). Endogenously expressed Trp1 is involved in store-mediated Ca^{2+} entry by conformational coupling in human platelets. J. Biol. Chem. *277*, 42157-42163.
12. Li, H.S., and Montell, C. (2000). TRP and the PDZ protein, INAD, form the core complex required for retention of the signalplex in *Drosophila* photoreceptor cells. J. Cell Biol. *150*, 1411-1422.
13. Tang, Y., Tang, J., Chen, Z., Trost, C., Flockerzi, V., Li, M., Ramesh, V., and Zhu, M.X. (2000). Association of mammalian trp4 and phospholipase C isozymes with a PDZ domain-containing protein, NHERF. J. Biol. Chem. *275*, 37559-37564.
14. Mery, L., Magnino, F., Schmidt, K., Krause, K.H., and Dufour, J.F. (2001). Alternative splice variants of hTrp4 differentially interact with the C-terminal portion of the inositol 1,4,5-trisphosphate receptors. FEBS Lett. *487*, 377-383.
15. Cioffi, D.L., Wu, S., and Stevens, T. (2003). On the endothelial cell I(SOC). Cell Calcium *33*, 323-336.
16. Zhang, S., Mizutani, A., Hisatsune, C., Higo, T., Bannai, H., Nakayama, T., Hattori, M., and Mikoshiba, K. (2003). Protein 4.1N is required for translocation of inositol 1,4,5-trisphosphate receptor type 1 to the basolateral membrane domain in polarized Madin-Darby canine kidney cells. J. Biol. Chem. *278*, 4048-4056.
17. Lockwich, T., Singh, B.B., Liu, X., and Ambudkar, I.S. (2001). Stabilization of cortical actin induces internalization of transient receptor potential 3 (Trp3)-associated caveolar Ca^{2+} signaling complex and loss of Ca2+ influx without disruption of Trp3-inositol trisphosphate receptor association. J. Biol. Chem. *276*, 42401-42408.
18. Singh, B.B., Liu, X., Tang, J., Zhu, M.X., and Ambudkar, I.S. (2002). Calmodulin regulates Ca(2+)-dependent feedback inhibition of store-operated Ca(2+) influx by interaction with a site in the C terminus of TrpC1. Mol. Cell *9*, 739-750.
19. Tang, J., Lin, Y., Zhang, Z., Tikunova, S., Birnbaumer, L., and Zhu, M.X. (2001). Identification of common binding sites for calmodulin and inositol 1,4,5-trisphosphate receptors on the carboxyl termini of trp channels. J. Biol. Chem. *276*, 21303-21310.
20. Patterson, R.L., van Rossum, D.B., Ford, D.L., Hurt, K.J., Bae, S.S., Suh, P.G., Kurosaki, T., Snyder, S.H., and Gill, D.L. (2002). Phospholipase C-gamma is required for agonist-induced Ca^{2+} entry. Cell *111*, 529-541.